Clinical predictors of response to single-agent immune checkpoint inhibitors in chemotherapy-pretreated non-small cell lung cancer

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Abstract. Single-agent immune checkpoint inhibitors (ICIs) are the standard option for chemotherapy-pretreated metastatic non-small cell lung cancer (NSCLC), however only a subset of patients responds to this treatment. The present study aimed at the development of a tool for personalized prediction of the efficacy of ICIs. The study included 181 epidermal growth factor receptor/anaplastic lymphoma kinase-negative patients with metastatic NSCLC receiving single-agent ICI in the second or later line of therapy. For the comparison, a total of 63 metastatic patients with NSCLC treated by chemotherapy were also analyzed. Multivariate analysis revealed that Eastern Cooperative Oncology Group performance status (ECOG PS) ≥2, never-smoking status and the baseline neutrophil-to-lymphocyte ratio (NLR) ≥4.3 were associated with reduced progression-free survival (PFS) and overall survival (OS) [ECOG PS: Hazard ratio (HR)=2.09; P=0.028 and HR=2.02; P=0.035, respectively; never-smoking: HR=3.53; P=0.007 and HR=1.80; P=0.004, respectively; NLR ≥4.3: HR=4.34; P<0.0001 and HR=4.89; P<0.0001 respectively]. Patients with an NLR <4.3, who had a favorable ECOG PS (0-1) and smoking history in the past, derived the utmost benefit from ICI [n=77; objective response rate (ORR)=35%; PFS and OS: 17.1 and 33.7 months, respectively]. The worst efficacy of ICI was observed in patients who had an NLR ≥4.3 coupled with poor ECOG PS and/or never-smoking status (n=38; ORR=8%; PFS=3.2 months and OS=7.2 months). The remaining patients belonged to the group with intermediate outcomes (n=66; ORR=17%; PFS and OS: 4.3 and 12.2 months, respectively). While combination of these factors was highly predictive for ICIs, it was not associated with outcomes of chemotherapy treatment. Easily available characteristics of the patients allow for highly accurate predictions of outcomes of single-agent ICI therapy in chemotherapy-pretreated NSCLC.

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Abbreviations: ALK, anaplastic lymphoma kinase; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR, epidermal growth factor receptor; HR, hazard ratio; ICIs, immune checkpoint inhibitors; IHC, immunohistochemical; irAEs, immune-related adverse events; NLR, neutrophil-to-lymphocyte ratio; NSCLC, non-small cell lung cancer; NSE, NLR, smoking status, ECOG; ORR, objective response rate; OS, overall survival; PD-L1, programmed cell death-ligand 1; PFS, progression-free survival; ROC, receiver operating characteristic

Key words: immune checkpoint inhibitors, non-small cell lung cancer, smoking, ECOG, neutrophil-to-lymphocyte ratio, peripheral blood biomarker

Introduction

Immune checkpoint inhibitors (ICIs) have become a standard of care for patients with metastatic non-small cell lung cancer (NSCLC) (1). Immunotherapy may be utilized alone in the first-line treatment if the NSCLC has a high level of PD-L1 expression (2). Single-agent ICIs can also be used in second and subsequent lines of NSCLC treatment, with different drugs having different requirements for programmed cell death-ligand 1 (PD-L1) status (3). In addition, ICIs can be administered upfront in combination with chemotherapy in both PD-L1-positive and -negative patients in the first-line settings (2). However, only a total of 20% of patients with NSCLC treated with immunotherapy demonstrate a durable response (1,2). There is an intensive search for reliable predictive markers allowing clinically meaningful selection of patients for the treatment with anti-PD-1/PD-L1 antibodies, thus avoiding unnecessary adverse events and decreasing financial burden (3).

Immunohistochemical (IHC) analysis of PD-L1 expression on tumor cells and/or tumor-infiltrating immune cells is the only companion test approved for ICI therapy of metastatic NSCLC (2). The indication was based on the results of the study KEYNOTE-024 (4), which showed improved progression-free survival (PFS) and overall survival (OS) using anti-PD-1 monotherapy compared with standard chemotherapy in previously untreated metastatic NSCLC expressing PD-L1 ≥50% (4). However, the predictive value of PD-L1 expression is controversial, as both positive and negative predictive value of this marker is relatively low (5). Other biomarkers, such as tumor mutation burden (TMB) or various gene expression signatures, have shown promise, however their use requires sophisticated laboratory tests and they have not been rigorously validated for reproducibility (6,7). Therefore, there is an unmet need for robust predictive markers for ICI efficacy in metastatic NSCLC.

Cancer-associated inflammation was suggested as one of the crucial factors interacting with immune surveillance (8,9). High neutrophil-to-lymphocyte ratio (NLR) is a well-known peripheral blood marker of systemic inflammation (10). Changes of the NLR may be an indicator of immune response in patients with various cancers, including metastatic NSCLC (10). Several studies have revealed that increased NLR has a negative predictive value in patients with metastatic NSCLC treated with ICIs (11,12). Baseline and on-treatment clinical parameters, such as smoking status, Eastern Cooperative Oncology Group performance status (ECOG PS) and immune-related adverse events (irAEs), may also serve as non-invasive and feasible predictive markers for ICIs. The use of these indicators alone proved to have insufficient reliability for personalizing ICI treatment (8). The accuracy of the prediction of the ICI response may be increased if other relevant parameters are taken into consideration (13). In the present study, the predictive role of clinical and laboratory characteristics of patients with NSCLC were evaluated and it was demonstrated that these are predictive for a long-term outcome of the ICI therapy.

Materials and methods

Patients and data collection. The present retrospective study included 181 patients with metastatic NSCLC without epidermal growth factor receptor (EGFR) mutations or anaplastic lymphoma kinase (ALK) rearrangements, who received single-agent ICI in the second or subsequent lines of therapy. The median age at diagnosis was 65 years, with a range of 34-86 years. The ICI cohort consisted of 129 males (71.3%) and 52 females (28.7%). Patients were treated according to standard clinical practice. These patients received at least two doses of standard therapeutic regimens (pembrolizumab, 200 mg intravenously once every 3 weeks; nivolumab, 3 mg/kg every 2 weeks or 480 mg every 4 weeks; atezolizumab, 1,200 mg intravenously once every 3 weeks). The therapy was performed until disease progression, unacceptable toxicity, or for up to two years. ICI predictive factors were also analyzed in the comparison group, composed of 63 patients with metastatic NSCLC without activating mutations in EGFR/ALK genes, who were treated by the first-line standard platinum-based chemotherapy and did not receive ICIs in subsequent treatment lines (comparison cohort). The median age was 63 years (range, 41-80 years). The latter cohort was collected to determine whether the analyzed predictive factors are ICI-specific.

All patients were treated between February 2017 and September 2022 at the Pavlov First Saint Petersburg State Medical University or at the N.P. Napalkov City Cancer Center (both at Saint Petersburg, Russia). The investigation was approved by the local Ethics Committee of Pavlov First Saint Petersburg State Medical University (approval no. 312-2022). The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and the ethical guidelines for medical and health research involving human subjects. All participants signed informed consent forms.

Inclusion criteria for both cohorts were as follows: Age, 18 years and older; ECOG PS, 0-3 (12); histologically confirmed NSCLC; absence of EGFR mutations and ALK translocations; and stage IV. The stage was classified based on the tumor node metastasis staging and the American Joint Committee on Cancer (8th edition, 2017) (14). Exclusion criteria for the ICI group were as follows: Previous immunotherapy; concomitant infection including human immunodeficiency virus or hepatitis; therapy by systemic steroids; and previous or ongoing autoimmune disease. Exclusion criteria for the chemotherapy cohort was the presence of ICIs in subsequent lines of therapy.

Baseline demographics (age, sex, body mass index and smoking status), clinical (ECOG PS, number of metastases and their sites), morphological (histology and PD-L1 expression) and treatment data (previous and subsequent type of treatment, line of therapy and type of anti-PD-1/PD-L1 agents) were assessed. The main characteristics of the patients are summarized in Table I. The tumor PD-L1 expression was assessed as part of standard clinical practice and was evaluated in 114 patients with NSCLC in the ICI cohort by two IHC kits: Dako PD-L1 clone 22C3 pharmDx (Agilent Technologies, Inc.) and Ventana PD-L1 clone SP142 (Ventana Medical Systems, Inc.).

The routine blood tests, including neutrophil and lymphocyte count for subsequent calculation of the ratio, were performed within one week before the start of therapy and after two cycles of treatment.

Tumor response was defined using the Immune Response Evaluation Criteria in Solid Tumors (iRECIST) (for ICIs cohort) and RECIST 1.1 (for comparison cohort) (15). irAEs, which occurred during the period of ICI administration, were also recorded. The severity of irAEs was graded using the criteria of the National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0 (16).

Statistical analysis. The endpoints of the study were objective response rate (ORR), PFS and OS. ORR was defined as a proportion of patients with complete and partial response according to iRECIST (for the ICI cohort) or RECIST 1.1 (for the comparison cohort). PFS was defined as the time from the start of ICI therapy to the progression of disease or censored at the date of the last patient contact or at the follow-up date. OS was defined as the time from the first cycle of ICI treatment until death or the date of last patient contact or the follow-up date.

Cut-off values were calculated using the receiver operating characteristic (ROC) analysis. Fisher's exact test and

Table I. Main baseline characteristics of patients receiving ICIs and chemotherapy.

Characteristics	ICIs cohort (n=181)	Comparison cohort (n=63)
Sex, n (%)		
Male	129 (71.3)	43 (68.3)
Female	52 (28.7)	20 (31.7)
Age (years), median (IQR), n (%)	65 (57-72)	63 (54-70)
<65	98 (54.1)	37 (58.7)
≥65	83 (45.9)	26 (41.3)
ECOG PS		
0-1	147 (81.2)	53 (84.1)
≥2	34 (18.8)	10 (15.9)
Smoking status		
Ever smoking	116 (64.1)	38 (60.3)
Never smoking	65 (35.9)	25 (39.7)
Histological type, n (%)		
Adenocarcinoma	95 (52.5)	26 (41.3)
Squamous cell carcinoma	82 (45.3)	36 (57.1)
Other NSCLCs	4 (2.2)	1 (1.6)
PD-L1 IHC expression		
<1%	30 (16.6)	0 (0.0)
≥1-49%	66 (36.5)	0 (0.0)
≥50%	18 (9.9)	63 (100.0)
No data	67 (37.0)	
Line of therapy, n (%)		
1	0 (0.0)	63 (100.0)
2	137 (74.7)	0 (0.0)
≥3	44 (24.3)	0 (0.0)
Type of therapy, n (%)		
Nivolumab	92 (50.8)	0 (0.0)
Pembrolizumab	76 (42.0)	0 (0.0)
Atezolizumab	13 (7.2)	0 (0.0)
Platinum-based doublet	0 (0.0)	63 (100.0)
ORR, n (%)	41 (23)	17 (27)
Median PFS, months (95% CI)	4.9 (4.2-5.6)	4.6 (4.0-5.9)
Median OS, months (95% CI)	13.7 (11.5-17.2)	10.4 (8.9-14.2)
Any irAEs, n (%)	71 (39)	0 (0.0)
Any grade ≥3 irAE, n (% of total irAEs)	12 (17)	
Thyroid dysfunction	25 (35)	
Skin reactions	17 (24)	
Pneumonitis	8 (11)	
Hepatitis	6 (8)	

ICI, immune checkpoint inhibitor; IQR, interquartile range; ECOG PS, Eastern Cooperative Oncology Group performance status; NSCLC, non-small lung cell cancer; PD-L1, programmed cell death-ligand 1; IHC, immunohistochemical; ORR, objective response rate; PFS, progression-free survival; OS, overall survival; CI, confidence interval; irAEs, immune-related adverse events.

chi-squared test were used to compare qualitative parameters and Mann-Whitney U test was applied to quantitative characteristics. PFS and OS were compared between the groups using a log-rank test with HR analysis and subsequent graphical visualization was performed using the Kaplan-Meier method. Multivariate Cox proportional hazard regression analysis was used to determine the relationship between survival and different potential predictive variables.

All statistical analyses were performed using GraphPad Prism (v.9.3.1; GraphPad Software, Inc.; Dotmatics). P<0.05 was considered to indicate a statistically significant difference.

Results

The median follow-up for patients receiving ICI was 13.6 months (95% CI, 11.5-16.1 months). The ORR was 41/181 (23%) (Table I). Median PFS in patients with NSCLC receiving ICI in the second or subsequent line of therapy approached 4.9 months (95% CI, 4.2-5.6 months) and median OS was 13.7 months (95% CI, 11.5-17.2 months) (Table I).

No significant association was observed between age, sex, ECOG PS, smoking history, histological tumor type, PD-L1 expression or line of the therapy and ORR (Table II) However, some clinical characteristics of the patients demonstrated statistically significant associations with PFS or OS. Median PFS was significantly longer in patients with good performance status (ECOG 0/1 vs. 2/3: 6.0 vs. 3.6 months, respectively; P<0.0001) (Fig. 1A). This association was also maintained for OS (15.8 vs. 9.8 months, P=0.003) (Fig. 2A). The median PFS and OS were significantly longer in ever-smokers compared with never-smokers [PFS: 11.3 vs. 4.0 months, P<0.0001 (Fig. 1B); OS: 16.6 vs. 9.9 months, P=0.008 (Fig. 2B)]. Presence of liver metastases was associated with shorter PFS [3.6 vs. 5.1 months, P=0.004 (Fig. 1C)], however this trend did not reach statistical significance for OS (P=0.084) (Fig. 2C). In addition, no relationship was observed between histological tumor type (squamous cell carcinoma vs. non-squamous cell carcinoma) and PFS (P=0.239) and OS (P=0.378) (data not shown).

PD-L1 expression status was known for 114 patients with patients with NSCLC treated by ICI therapy. The optimal cut-off value for tumor PD-L1 expression was determined as ≥1% for both PFS and OS. There was no statistically significant relationship between PD-L1 expression ≥1% and longer PFS (5.6 vs. 4.1 months, HR 1.25; 95% CI, 0.89-1.83; P=0.216) (Fig. 3A) and OS (14.6 vs. 10.9 months; HR 1.33; 95% CI, 0.90-1.99; P=0.151) (Fig. 4A). Furthermore, stratification into three categories of PD-L1 expression (<1%, ≥1-49% and ≥50%) did not reveal a significant association with PFS (P=0.354) and OS (P=0.413) (data not shown).

irAEs were observed in 71/181 (39%) patients with NSCLC receiving second or subsequent lines of ICI (Table I). Grade ≥3 toxicity was detected in 12/71 patients (17%) (Table I). The most common irAEs were thyroid dysfunction [25/71 (35%)], skin reactions [17/71 (24%)], pneumonitis [8/71 (11%)] and hepatitis [6/71 (8%)] (Table I). The presence of irAEs was associated with prolonged OS (18.5 vs. 11.3 months, P=0.011) (Fig. 2D), but not with increased PFS (P=0.098) (Fig. 1D). In addition, no significant association was observed between irAEs and ORR (Table II).

Table II. ORRs according to main characteristics in patients receiving ICIs.

Characteristics	ORR, % (N)	P-value
Age (<65 vs. ≥65)	24 (24/98) vs. 20 (17/83)	0.594
Sex (male vs. female)	24 (31/129) vs. 19 (10/52)	0.560
ECOG PS (0/1 vs. ≥2)	25 (37/147) vs. 12 (4/34)	0.113
Smoking (former/active vs. never)	26 (30/116) vs. 17 (11/65)	0.198
Liver metastasis (no vs. yes)	24 (36/153) vs. 14 (4/28)	0.329
Histology (non-squamous vs. squamous)	26 (25/95) vs. 19 (16/86)	0.286
irAEs (yes vs. no)	28 (20/71) vs. 19 (21/110)	0.203
NLR (<4.3 vs. ≥4.3)	27 (34/124) vs. 12 (7/57)	0.034
NLR after two cycles (<4.5 vs. ≥4.5)	27 (31/116) vs. 15 (10/65)	0.098
NLR dynamic changes (<24% vs. ≥24%)	26 (28/106) vs. 19 (14/75)	0.284
NSE score		0.002
Group 1	35 (27/77)	
Group 2	17 (11/66)	
Group 3	8 (3/38)	

ORR, objective response rate; ICIs, immune checkpoint inhibitors; ECOG PS, Eastern Cooperative Oncology Group performance status; irAEs, immune-related adverse events; NLR, neutrophil-to-lymphocyte ratio; NSE, NLR, smoking status, ECOG.

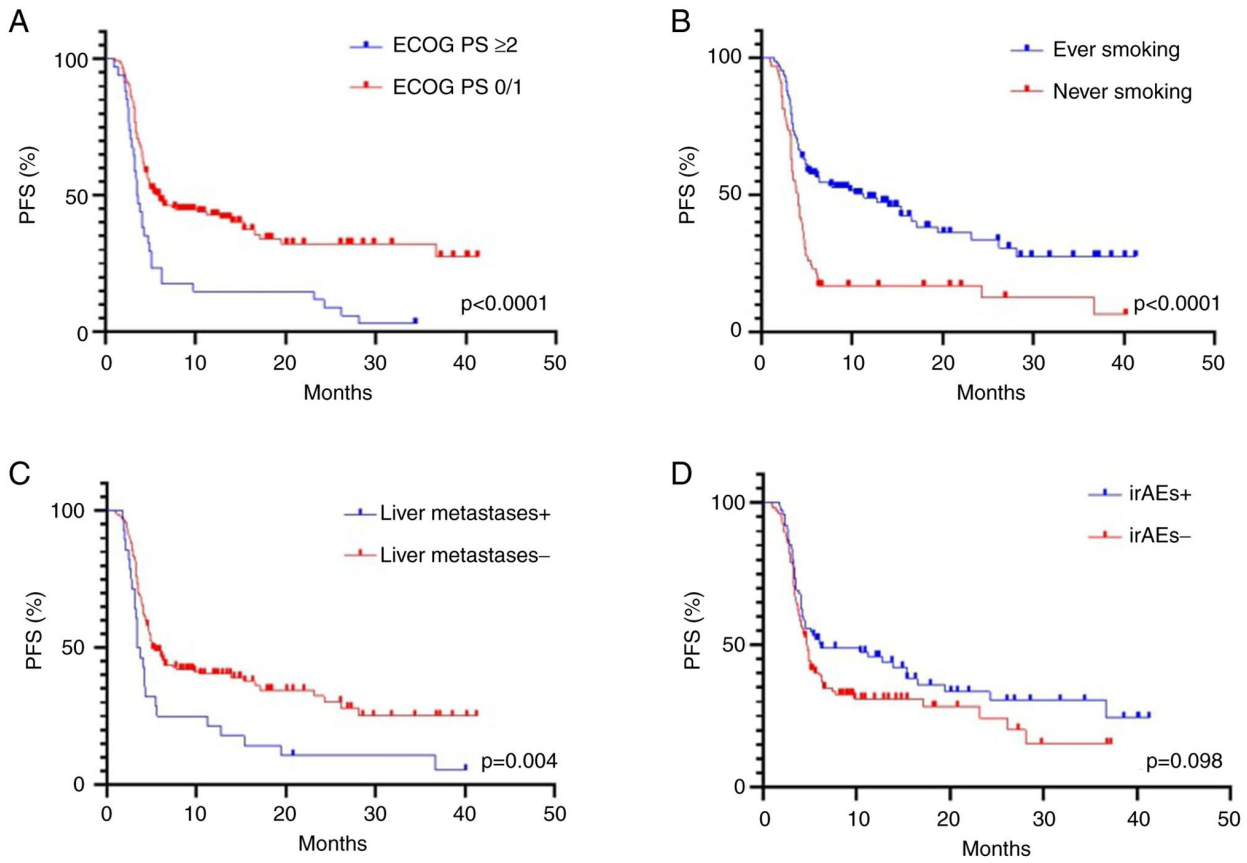

Figure 1. Kaplan-Meier curves for PFS according to (A) ECOG PS, (B) smoking status, (C) liver metastases and (D) irAEs in patients with metastatic non-small cell lung cancer receiving second and subsequent lines of immunotherapy. PFS, progression-free survival; ECOG PS, Eastern Cooperative Oncology Group performance status; irAEs, immune-related adverse events.

NLR was significantly associated with the outcome of ICI therapy. The highest predictive value was observed for the threshold equal to 4.3, as determined by the ROC analysis. PFS was significantly shorter in patients with baseline NLR ≥4.3 compared with patients with NLR <4.3: (3.2 vs. 15.4 months, P<0.0001) (Fig. 3B). The median of OS was also

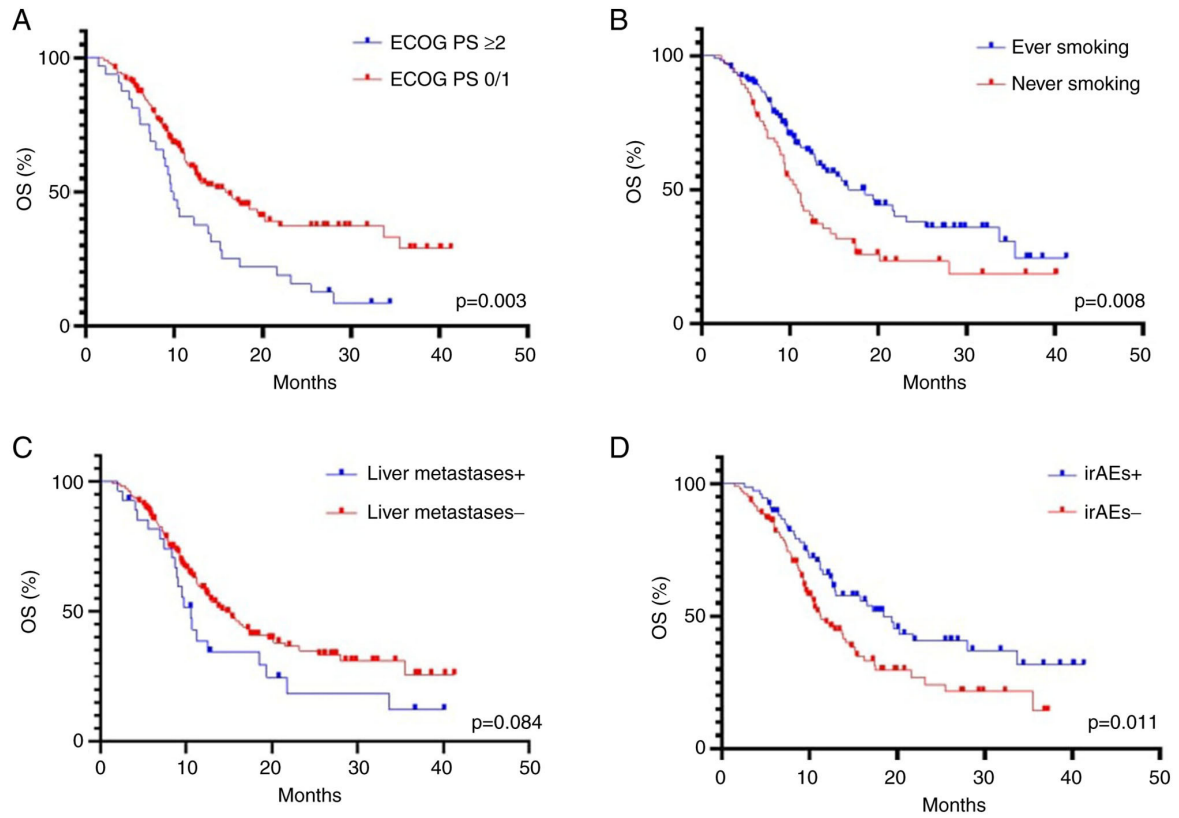

Figure 2. Kaplan-Meier curves for OS according to (A) ECOG PS, (B) smoking status, (C) liver metastases and (D) irAEs in patients with metastatic non-small cell lung cancer receiving second and subsequent lines of immunotherapy. OS, overall survival; ECOG PS, Eastern Cooperative Oncology Group performance status; irAEs, immune-related adverse events.

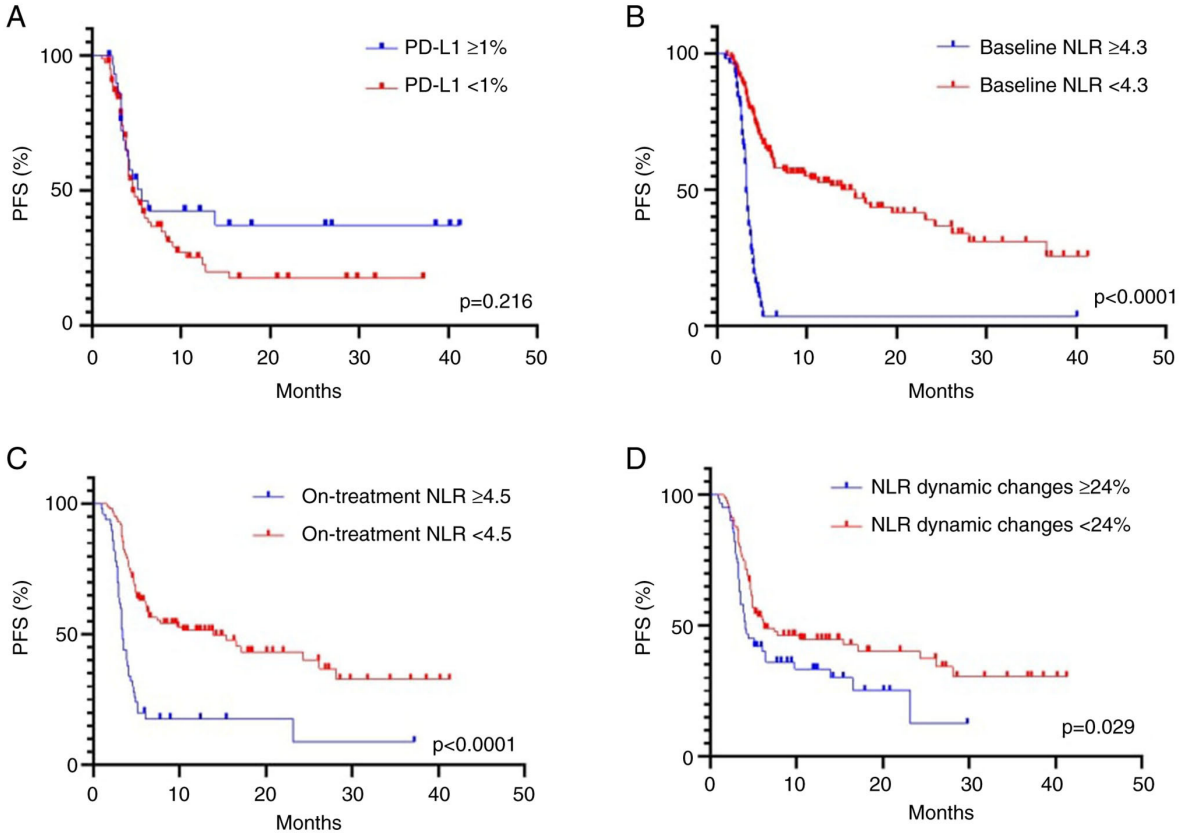

Figure 3. Kaplan-Meier curves for PFS according to (A) PD-L1 expression, (B) baseline NLR, (C) NLR after two cycles and (D) NLR dynamic changes in patients with metastatic non-small cell lung cancer receiving second and subsequent lines of immune checkpoint inhibitors. PFS, progression-free survival; PD-L1, programmed cell death-ligand 1; NLR, neutrophil-to-lymphocyte ratio.

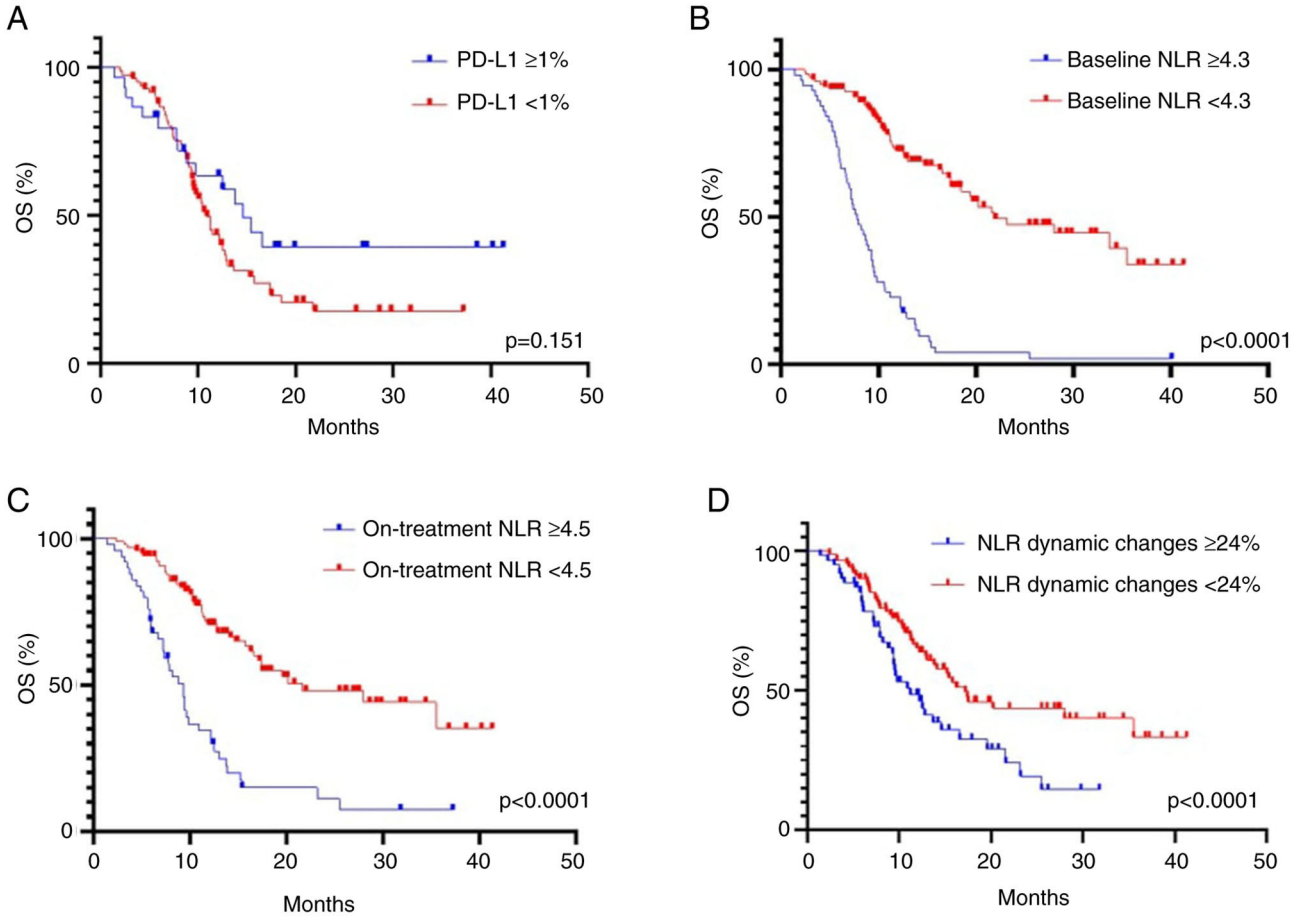

Figure 4. Kaplan-Meier curves for OS according to (A) PD-L1 expression, (B) baseline NLR, (C) NLR after two cycles and (D) NLR dynamic changes in patients with metastatic non-small cell lung cancer receiving second and subsequent lines of immune checkpoint inhibitors. OS, overall survival; PD-L1, programmed cell death-ligand 1; NLR, neutrophil-to-lymphocyte ratio.

evidently shorter in patients with the NLR ≥4.3 compared with the NLR <4.3 group (7.8 vs. 21.8 months, P<0.0001) (Fig. 4B). The ORR was significantly higher in patients with baseline NLR <4.3 compared with patients with NLR ≥4.3: 27 and 12%, respectively (P=0.034) (Table II).

The cut-off value for NLR after two cycles was determined as ≥4.5 for monitoring ICI efficacy. The NLR ≥4.5 after two cycles was a predictor of worse PFS (3.4 vs. 13.9 months, P<0.0001) (Fig. 3C) and OS (9.3 vs. 21.6 months, P<0.0001) (Fig. 4C). It was further investigated whether the dynamic change of NLR (the difference between the ratio after two cycles and baseline value) observed during ICI therapy had a predictive value. The optimal cut-off level of the dynamic change of NLR was an increase of ≥24%. The NLR dynamic changes of ≥24% were associated with shorter PFS (4.0 vs. 6.3 months, P=0.029) (Fig. 3D) and OS (11.2 vs. 17.2 months, P<0.0001) (Fig. 4D). The improvement of NLR during ICI exposure tended to indicate a better outcome, however none of the thresholds determined by ROC analysis produced statistically significant associations. In addition, no significant association was observed between NLR after two cycles, NLR dynamic changes and the ORR (Table II). Univariate analysis of the patient data demonstrated that ECOG PS ≥2 (HR, 1.98; 95% CI, 1.30-2.93; P=0.001), never smoking (HR, 1.91; 95% CI, 1.38-2.82; P=0.001), presence of liver metastasis (HR, 1.42; 95% CI, 1.07-2.31; P=0.026), NLR ≥4.3 (HR, 4.79; 95% CI, 3.24-7.07; P<0.0001) and NLR after two cycles of ICI (HR, 2.09; 95% CI, 1.43-3.01; P=0.0001) were significantly associated with worse PFS (Table III). In multivariate analysis, only ECOG PS ≥2 (HR, 2.09; 95% CI, 1.09-4.07; P=0.028), never smoking status (HR, 3.53; 95% CI, 2.07-9.29; P=0.007) and baseline NLR ≥4.3 (HR, 4.34; 95% CI, 2.65-7.03; P<0.0001) retained significance for decreased PFS (Table III).

The univariate analysis for OS demonstrated that ECOG PS ≥2 (HR, 2.09; 95% CI, 1.35-3.16; P=0.001), never smoking (HR, 1.82; 95% CI, 1.23-2.69; P=0.003), absence of irAEs (HR, 1.69; 95% CI, 1.14-2.54; P=0.011), baseline NLR ≥4.3 (HR, 5.07; 95% CI 3.58-8.09; P<0.0001), NLR after two cycles of the therapy (HR 2.67; 95% CI, 1.78-3.96; P<0.0001) and NLR dynamic changes ≥24% (HR, 1.66; 95% CI, 1.11-2.46; P=0.012) were negative predictive factors (Table IV). However, in multivariate analysis only ECOG PS ≥2 (HR, 2.02; 95% CI, 1.06-3.91; P=0.035), never smoking (HR, 1.80; 95% CI, 1.21-2.68; P=0.004) and baseline NLR ≥4.3 (HR, 4.89; 95% CI, 3.16-7.62; P<0.0001) retained significance for OS (Table IV).

Baseline prognostic score. In multivariate analysis, the independent predictors of both worse PFS and OS were pretreatment NLR ≥4.3, non-smoking status and ECOG PS≥2. Based on the data, a baseline prognostic NLR, smoking status, ECOG (NSE) score named after the first letters of the included markers was developed (NLR at baseline

Table III. Univariate and multivariate analyses for PFS using Cox proportional hazards regression model in ICIs cohort.

	Univariate analysis		Multivariate analysis	
Characteristics	HR (95% CI)	P-value	HR (95% CI)	P-value
PFS				
Age	1.00 (0.98-1.02)	0.962	-	-
Sex (male vs. female)	0.73 (0.49-1.19)	0.249	-	-
BMI	0.99 (0.94-1.03)	0.605		
ECOG PS (≥2 vs. 0/1)	1.98 (1.30-2.93)	0.001	2.09 (1.09-4.07)	0.028
Smoking (never vs. former/active)	1.91 (1.38-2.82)	0.001	3.53 (2.07-9.29)	0.007
Liver metastasis (yes vs. no)	1.42 (1.07-2.31)	0.026	1.55 (0.92-2.54)	0.083
Brain metastasis (yes vs. no)	0.89 (0.44-1.62)	0.731	-	-
Bone metastasis (no vs. yes)	0.91 (0.58-1.50)	0.709	-	-
Metastatic sites (≥ 2 vs. <2)	1.12 (0.76-1.65)	0.419	-	-
Histology (squamous vs. adenocarcinoma)	1.19 (0.84-1.72)	0.333	-	-
Previous radiotherapy (no vs. yes)	1.22 (0.69-2.34)	0.519	-	-
Immunotherapy [anti-PD-1 (pembrolizumab/ nivolumab) vs. anti-PD-L1 (atezolizumab)]	1.09 (0.66-1.71)	0.718	-	-
Line of therapy (2L vs. 3L+)	1.28 (0.80-1.92)	0.305	-	-
NLR (≥4.3 vs. <4.3)	4.79 (3.24-7.07)	<0.0001	4.34 (2.65-7.03)	<0.0001
NLR after two cycles (≥4.5 vs. <4.5)	2.09 (1.43-3.01)	0.0001	1.27 (0.84-1.91)	0.252
NLR dynamic changes (≥24% vs. <24%)	1.23 (0.85-1.76)	0.259	-	-
irAEs (no vs. yes)	1.36 (0.95-1.97)	0.099	-	-

PFS, progression-free survival; ICIs, immune checkpoint inhibitors; HR, hazard ratio; CI, confidence interval; BMI, body mass index; ECOG PS, Eastern Cooperative Oncology Group performance status; PD-L1, programmed cell death-ligand 1; NLR, neutrophil-to-lymphocyte ratio; irAEs, immune-related adverse events; NSE, NLR, smoking status.

≥4.3, 2 points; non-smoking status, 1 point; and ECOG ≥2, 1 point) (Table V). NSE score categorized three groups of patients with NSCLC depending on the outcome of ICI therapy: Good (group 1, 0 points); intermediate (group 2, 1-2 points) and poor (group 3, ≥3 points) (Table V). Among patients receiving ICIs, 77 patients (43%) belonged to the NSE group 1, 66 (36%) to group 2 and 38 (21%) to group 3. The ORR in good, intermediate and poor prognostic groups was 35, 17 and 8%, respectively (P=0.002) (Table II). The median PFS for group 1 was 17.1 months, and for groups 2 and 3 it was 4.3 and 3.2 months, respectively (P<0.0001) (Fig. 5A). The median OS was 33.7, 12.2 and 7.2 months, respectively (P<0.0001) (Fig. 5B). Strong differences between these groups retained significance upon multivariate analysis (Table VI). In addition, no significant differences were revealed between the type of ICI (pembrolizumab, nivolumab or atezolizumab) and the endpoints of the study in each prognostic group (data not shown).

Chemotherapy cohort. Median follow-up was 15.3 months (95% CI, 11.3-20.2 months) in the chemotherapy cohort. The ORR was 27% in this group of patients. Median PFS and OS were 4.6 months (95% CI, 4.0-5.9 months) and 10.4 months (95% CI, 8.9-14.2 months), respectively (Table I).

No significant association was observed between clinical parameters, peripheral blood markers (baseline NLR, NLR after two cycles and NLR dynamic changes) and treatment efficacy (ORR, PFS and OS) in patients receiving chemotherapy (Table VII).

It was also addressed whether NSE score as aforementioned is predictive for benefit from cytotoxic drugs. In the chemotherapy cohort according to the NSE score, a total of 20 patients (32%) were assigned to group 1, 27 (42.9%) to group 2 and 16 (25%) to group 3 (data not shown). No difference was observed in terms of response rate according to scoring groups. The PFS and OS were similar in these groups [PFS: 4.8, 4.1 and 4.6 months, respectively; P=0.226 (Fig. 5C); OS: 12.8, 10.2 and 10.1 months, respectively; P=0.389 (Fig. 5D)].

Discussion

In the present study the predictive role of certain clinical characteristics and peripheral blood markers on efficacy to ICIs was investigated. ECOG performance status, smoking history, presence of liver metastasis, irAEs and NLR demonstrated associations with outcomes in metastatic patients with NSCLC receiving single-agent ICI in second and subsequent lines of therapy. Additionally, a combination of NLR, ECOG and smoking history yielded an accurate prediction of ICI therapy outcome. This combination was not predictive in the patients, who received chemotherapy without subsequent ICI administration. The comparison of these two groups is compromised by the fact that ICI treatment was applied in second or subsequent lines of therapy, while cytotoxic drugs were utilized upfront.

Table IV. Univariate and multivariate analyses for OS using Cox proportional hazards regression model in ICIs cohort.

	Univariate analysis		Multivariate analysis	
Characteristics	HR (95% CI)	P-value	HR (95% CI)	P-value
OS				
Age	1.01 (0.99-1.03)	0.417	-	-
Sex (male vs. female)	0.78 (0.52-1.18)	0.234	-	-
BMI	0.97 (0.92-1.02)	0.218	-	-
ECOG PS (≥2 vs. 0/1)	2.09 (1.35-3.16)	0.001	2.02 (1.06-3.91)	0.035
Smoking (never vs. former/active)	1.82 (1.23-2.69)	0.003	1.80 (1.21-2.68)	0.004
Liver metastasis (yes vs. no)	1.48 (0.88-2.36)	0.119	-	-
Brain metastasis (yes vs. no)	0.91 (0.43-1.69)	0.780	-	-
Bone metastasis (no vs. yes)	0.81 (0.50-1.37)	0.407	-	-
Metastatic sites (≥2 vs. <2)	1.12 (0.76-1.65)	0.559	-	-
Histology (squamous vs. adenocarcinoma)	1.30 (0.73-2.59)	0.409	-	-
Previous radiotherapy (no vs. yes)	1.45 (0.79-2.98)	0.266	-	-
Immunotherapy [anti-PD-1 (pembrolizumab/ nivolumab) vs. anti PD-L1 (atezolizumab)]	0.89 (0.49-1.50)	0.629	-	-
Line of therapy (2L vs. 3L+)	1.23 (0.72-1.98)	0.419	-	-
NLR baseline (≥4.3 vs. <4.3)	5.07 (3.58-8.09)	<0.0001	4.89 (3.16-7.62)	<0.0001
NLR after two cycles (≥4.5 vs. <4.5)	2.67 (1.78-3.96)	<0.0001	1.41 (0.58-2.93)	0.398
NLR dynamic changes (≥24% vs. <24%)	1.66 (1.11-2.46)	0.012	1.59 (0.97-2.63)	0.064
irAEs (no vs. yes)	1.69 (1.14-2.54)	0.011	1.63 (0.91-2.82)	0.088

OS, overall survival; ICIs, immune checkpoint inhibitors; HR, hazard ratio; CI, confidence interval; BMI, body mass index; ECOG PS, Eastern Cooperative Oncology Group performance status; PD-L1, programmed cell death-ligand 1; NLR, neutrophil-to-lymphocyte ratio; irAEs, immune-related adverse events.

Table V. NSE predictive scoring system and risk stratification.

Predictive factor	Value	Points
NLR at baseline	≥4.3	2
	<4.3	0
Smoking status	Never	1
	Ever	0
ECOG PS	≥2	1
	0-1	0

Predictive groups: Good (group 1), 0 points; intermediate (group 2), 1-2 points; and poor (group 3), ≥3 points. NSE, NLR, smoking status, ECOG; NLR, neutrophil-to-lymphocyte ratio; ECOG PS, Eastern Cooperative Oncology Group performance status.

However, it is considered that this approach is optimal to minimize the role of confounding factors. Notably, only a minority of patients with NSCLC receive ICI therapy without chemotherapy in the first line and the selection of these cases is based on strict criteria, thus, it was not possible to collect a substantial number of subjects in this setting. However, the responses of patients with NSCLC to chemotherapy in the second or third lines of treatment are usually minimal, thus this analysis was intentionally limited to the first line platinum-based doublets.

The investigation did not demonstrate that PD-L1 expression status was significant, although some patients did not undergo PD-L1 testing and two different IHC assays were used for the PD-L1 analysis. These results were consistent with studies, which utilized single-agent nivolumab or atezolizumab after failure of cytotoxic therapy (17,18). However, a clinical trial revealed that patients with NSCLC with PD-L1 expression in >1% of tumor cells had improved treatment outcomes (19). A meta-analysis involving 3,688 patients from seven randomized trials, who were subjected to the second or subsequent lines of therapy and were evaluated for OS, revealed that ICIs outperform cytotoxic drugs across all PD-L1 expression subgroups (20). At the same time, the most marked effect from ICIs was observed for NSCLCs expressing PD-L1 in >50% of tumor cells (20).

In a number of studies, the immunotherapy survival benefit was shown to be associated with the anatomic location of metastasis (21). The liver has an immunosuppressive microenvironment, thus the presence of metastases in this organ may influence the efficacy of the ICI (22). A recent meta-analysis has shown that metastatic involvement of the liver was associated with worse PFS in patients with NSCLC, although it did not result in shorter OS (23). The results of the present study provided some support for the aforementioned findings. Another clinical parameter, that may serve as a potential predictor of ICI efficacy, is the presence of irAEs. The rationale is that irAEs are caused by hyperactivation of

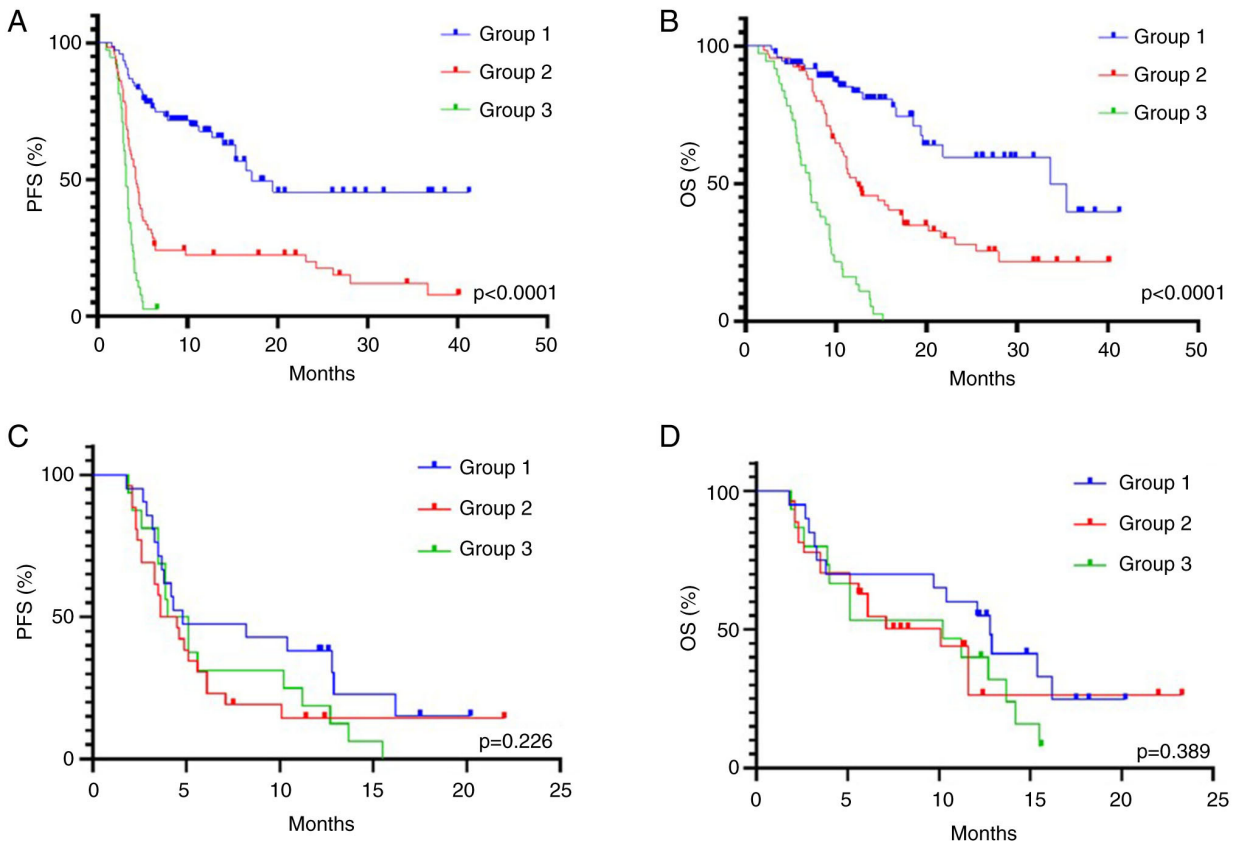

Figure 5. Kaplan-Meier curves for (A) PFS and (B) OS according to the NSE score in patients with metastatic non-small cell lung cancer receiving second and subsequent lines of immune checkpoint inhibitors. (C) PFS and (D) OS according to the NSE groups in the chemotherapy cohort. PFS, progression-free survival; OS, overall survival; NSE, NLR, smoking status, ECOG.

the immune system during ICI therapy and, hence, are indirectly associated with antitumor response (24). Similarly, to previous studies (25,26), the presence of irAEs in the data of the present study was associated with prolonged OS. However, this association was not retained upon multivariate analysis.

The contribution of ECOG performance status in determining treatment outcome is well established. Patients with poor overall condition usually have compromised 'defense' mechanisms. In addition, the development of antitumor immune response may take time, which is another factor affecting outcomes in patients with short life expectancy (27). ECOG PS ≥2 was an independent predictor of short PFS and OS in the present study, which is consistent with other studies (20,28).

Smoking history is a particularly relevant predictive marker for ICI response in patients with NSCLC. Cigarette consumption is associated with high TMB and consequently, increased production of tumor neoantigens (29). The data of the present study on improved ICI treatment outcomes in smokers are consistent with the results of meta-analysis and other individual studies (30-32).

NLR is a surrogate marker of chronic inflammation (33). Neutrophils in the peripheral blood represent precursors of immunosuppressive cells (tumor-associated neutrophils and granulocytic myeloid-derived suppressor cells) in the tumor microenvironment, which promote tumor progression (11,34). In turn, lymphocytes are responsible for cellular immune response (11). A high NLR ratio is a predictor of worse prognosis in NSCLC, regardless of the treatment type. Increased NLR may predict for poor outcomes of chemotherapy (35), however, the data of the present study did not confirm this association. In addition, NLR was predictive for poor efficacy of immune therapy, which is in agreement with previous research (36). It was also demonstrated that a high level of NLR before and after two cycles of ICI and the dynamic increase of this ratio during the ICI treatment, were associated with worse survival outcomes. Similar results were produced by several other studies (12,37,38). The elimination of immunosuppressive derivatives of peripheral blood neutrophils appears to be a promising strategy for overcoming the resistance to ICIs (34). In accordance with this hypothesis, therapeutic modulators of immunosuppressive neutrophils increased the efficacy of ICI in preclinical models (34). However, only a limited number of these combinations have reached clinical trials and are currently under investigation (39-41).

The combined assessment of different markers in a predictive score, rather than using a single predictive marker, may improve identification of patients who are most likely to benefit from ICI therapy. Numerous predictive ICI-related scores have been studied in patients with advanced NSCLC, such as EPSILoN, ALI, LIPI, iSEND, LIPS-3 and combined NLR-TMB score (8,9,14,20,42,43). All these indices are included in the NLR. However, some of them included laboratory markers, such as TMB, PD-L1 and LDH, which are not routinely studied in a number of centers for ICI therapy in second and subsequent lines of treatment in patients with NSCLC.

Table VI. Multivariate analysis for PFS and OS according to NSE score groups.

	PFS		OS	
NSE score	HR (95% CI)	P-value	HR (95% CI)	P-value
Group 1	1 (Reference)		1 (Reference)	
Group 2	3.09 (1.94-4.99)	<0.0001	2.61 (1.49-4.32)	0.0003
Group 3	7.31 (3.95-11.68)	<0.0001	6.99 (3.89-10.03)	<0.0001

NSE, NLR, smoking status, ECOG; PFS, progression-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval.

Table VII. Univariate analysis for PFS and OS using Cox proportional hazards regression model in a chemotherapy cohort.

	PFS		OS	
Characteristics	HR (95% CI)	P-value	HR (95% CI)	P-value
Age	1.09 (0.86-1.34)	0.545	1.04 (0.86-1.32)	0.704
Sex (male vs. female)	1.15 (0.66-2.32)	0.694	1.09 (0.46-1.74)	0.781
ECOG PS (≥2 vs. 0/1)	1.25 (0.85-1.75)	0.232	1.42 (0.90-1.81)	0.131
Smoking (never vs. former/active)	0.74 (0.44-1.30)	0.292	0.77 (0.58-1.21)	0.176
Liver metastasis (yes vs. no)	1.44 (0.70-2.94)	0.319	1.31 (0.68-2.98)	0.476
Bone metastasis (no vs. yes)	0.78 (0.63-1.42)	0.345	0.64 (0.58-1.63)	0.254
Metastatic sites (≥2 vs. <2)	1.32 (0.81-1.86)	0.249	1.22 (0.76-2.14)	0.312
Histology (squamous vs. adenocarcinoma)	1.16 (0.67-1.98)	0.582	1.20 (0.76-2.04)	0.483
NLR baseline (≥3.1 vs. <3.1)	1.30 (0.86-1.91)	0.250	1.54 (0.88-2.82)	0.151
NLR after two cycles (≥3.9 vs. <3.9)	1.27 (0.77-2.11)	0.346	1.37 (0.84-2.32)	0.219
NLR dynamic changes (≥15% vs. <15%)	1.64 (0.60-3.45)	0.344	1.28 (0.68-2.38)	0.476
NSE score (group 1 vs. group 2/3)	1.28 (0.83-1.98)	0.276	1.25 (0.76-2.02)	0.387

PFS, progression-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; NLR, neutrophil-to-lymphocyte ratio; NSE, NLR, smoking status, ECOG.

A new baseline prognostic score named NSE was developed. It is based on markers that were independent predictors of survival outcomes in multivariate analysis. The advantage of the approach is the simplicity of it, as it is based on easily accessible characteristics of the patients. In brief, the data of the present study suggested that patients with an NLR ratio <4.3, who have good performance status and history of smoking, are the most likely to derive benefit from ICI therapy. By contrast, patients with an NLR ≥4.3 coupled with poor ECOG and/or lack of tobacco exposure, are highly unlikely to respond to ICI. The remaining subjects consist of the group of intermediate ICI therapy outcome. Notably, this reasoning is specific for immunotherapy, as the same scoring was not predictive for patients receiving cytotoxic drugs. The limitations of this investigation were the retrospective nature of the study and the absence of other routine blood tests, including absolute lymphocyte count and platelet-lymphocyte ratio, which were not considered in the data analysis.

In conclusion, the independent predictive factors for short PFS and OS, such as a baseline NLR ≥4.3, non-smoking status and ECOG PS ≥2 were demonstrated in the present study. In addition, the developed new NSE score based on these markers may assist the decision-making for NSCLC immunotherapy in second and subsequent line settings. However, the score requires validation in a prospective study.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

AAM was responsible for the development of the research design, review of publications on the topic of the article, analysis of the data obtained, design of illustrative material, statistical analysis and article writing. FVM was responsible for the development of the research design, methodology and analysis of the data obtained. TEE, APO, KAO, MAU, SVOd, IVC and AMD were involved in curation of patients and data collection. ALA, ENI and SVOr were responsible for conception and design development, as well as scientific editing and research management. AAM and SVOr confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The study was approved (approval no. 312 2022) by the Ethics Committee of Pavlov First Saint Petersburg State Medical University (Saint Petersburg, Russia). All participants signed informed consent forms.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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